

Neuro-Pulmonotoxic Effects of Dichlorvos, Dimethoate, and Cypermethrin in Postpartum Rats

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ABSTRACT

Pesticide mixtures are widely used in agriculture, yet their cumulative health impacts remain poorly defined, particularly in physiologically vulnerable populations. This study investigated the biochemical and histopathological alterations induced by dichlorvos, dimethoate, and cypermethrin—individually and in mixtures—in the brain and lungs of postpartum female Wistar albino rats. Animals were exposed to sprayed formulations and assessed for reduced glutathione (GSH), acid phosphatase (ACP), alkaline phosphatase (ALP), and tissue pathology. Significant oxidative stress was evident through marked GSH depletion. In brain tissue, control rats recorded ~8.5 µM GSH, whereas dichlorvos and dimethoate reduced levels to ~6.2 µM and ~5.9 µM, respectively ($p < 0.05$). The greatest depletion occurred with the triple mixture (~4.3 µM). In the lungs, baseline GSH (~27 µM) declined to ~18 µM under dichlorvos or cypermethrin, ~13 µM with dimethoate, and as low as ~6 µM in the triple mixture ($p < 0.05$). Lysosomal stress was confirmed by increased ACP activity: brain ACP rose from ~0.03 U/mg protein in controls to ~1.20 U/mg in the triple mixture, while lung ACP reached ~0.85 U/mg under the same condition. Conversely, ALP activity was suppressed, dropping from ~1.25 U/mg protein in control brain to ~0.18 U/mg in the triple mixture, and from ~1.18 to ~0.48 U/mg in lung tissue. Histopathological findings corroborated biochemical data, revealing neuronal degeneration, oedema, vascular congestion, bronchiolar ulceration, and necrosis, with the most severe lesions in the triple-exposed group. These results demonstrate that combined pesticide exposure exerts additive or synergistic neuro- and pulmonotoxic effects in postpartum rats, underscoring heightened maternal vulnerability and the need for stricter regulation of pesticide mixtures.

Keywords: Pesticide Mixtures; Dichlorvos; Dimethoate; Cypermethrin; Oxidative Stress; Glutathione Depletion; Enzyme Biomarkers; Acid Phosphatase; Alkaline Phosphatase; Histopathology; Neurotoxicity; Pulmonotoxicity.

1. Introduction

Pesticides are extensively employed in agricultural practices to enhance crop yield and pest control, yet their widespread use raises significant concerns about environmental persistence and human health impacts. Among these, organophosphates such as dichlorvos and dimethoate, and pyrethroids such as cypermethrin, are among the most frequently applied insecticides in developing countries, including Nigeria, owing to their affordability and effectiveness [1,2]. However, increasing evidence highlights that exposure to these agents, even at sub-lethal levels, is associated with oxidative stress, enzymatic disruption, and tissue injury in mammals [3,4].

Organophosphates act primarily by irreversibly inhibiting acetylcholinesterase, resulting in neuronal hyperexcitation and cytotoxic stress [5]. Beyond neurotoxicity, they generate reactive oxygen species (ROS) that deplete endogenous antioxidants such as reduced glutathione (GSH), impair membrane-bound enzyme systems, and compromise cellular homeostasis [6]. Pyrethroids, exemplified by cypermethrin, though considered relatively safer, disrupt sodium channel kinetics and also trigger oxidative and lysosomal stress [7,8]. Importantly, these pesticides are often applied in mixtures, either intentionally for synergistic pest control or inadvertently through environmental contamination. Co-exposures may elicit additive or synergistic toxicological outcomes, leading to exacerbated biochemical and histopathological changes compared to single pesticide exposures [9,10].

The postpartum period represents a particularly vulnerable physiological state characterised by hormonal shifts, altered immune responses, and heightened metabolic demand, all of which increase susceptibility to oxidative

insults [11,12]. Yet, limited studies have assessed the cumulative impact of pesticide mixtures in postpartum females, despite their frequent occupational and domestic exposure in agricultural communities.

This study therefore investigates the biochemical and histopathological alterations induced by dichlorvos, dimethoate, and cypermethrin—individually and in mixtures—in the brain and lungs of postpartum female Wistar rats. By evaluating reduced glutathione (GSH), acid phosphatase (ACP), alkaline phosphatase (ALP), and tissue architecture, the research provides insights into the mechanistic basis of neuro- and pulmonotoxicity associated with combined pesticide exposure in a physiologically sensitive model.

1.1. Study Objectives

The objectives of this study are to:

1. Evaluate the effects of dichlorvos, dimethoate, and cypermethrin—individually and in mixtures—on antioxidant status in postpartum female Wistar rats.
2. Determine alterations in enzymatic biomarkers (acid phosphatase and alkaline phosphatase) following pesticide exposure.
3. Assess histopathological changes in brain and lung tissues induced by individual and combined pesticide exposures.
4. Compare the severity of toxicological responses between single, double, and triple pesticide mixtures.
5. Establish the implications of combined pesticide exposure for neurotoxicity and pulmonotoxicity in the postpartum physiological state.
6. Provide scientific evidence to support stricter regulation of pesticide mixture use and exposure.

2. Materials and Methods

2.1. Animals

Twenty-four healthy postpartum female Wistar albino rats (*Rattus norvegicus*), weighing 180–200 g, were obtained from the Animal House, Department of Environmental Management and Toxicology, Federal University of Petroleum Resources, Effurun, Nigeria. Animals were housed in standard cages under controlled conditions (temperature 23 ± 2 °C; relative humidity 50–60%; 12 h light/12 h dark cycle). Rats had free access to commercial pellet diet and clean tap water. All procedures were conducted in accordance with institutional ethical guidelines for animal experimentation and were approved by the University Ethical Review Committee (Approval No.: EMT/2025/012).

2.2. Chemicals

Technical-grade dichlorvos (98%), dimethoate (97%), and cypermethrin (95%) were purchased from Sigma-Aldrich (USA). Stock solutions were prepared in distilled water and administered via oral gavage. All other reagents and chemicals were of analytical grade.

(a) Experimental Design and Treatment Protocol

The postpartum female Wistar albino rats were randomly assigned into eight groups ($n = 4$ per group) as follows:

- **Group A (Control):** exposed to water spray.
- **Group B:** exposed to dichlorvos.
- **Group C:** exposed to dimethoate.
- **Group D:** exposed to cypermethrin.
- **Group E:** exposed to dichlorvos and dimethoate.
- **Group F:** exposed to dichlorvos and cypermethrin.
- **Group G:** exposed to dimethoate and cypermethrin.
- **Group H:** exposed to dichlorvos, dimethoate, and cypermethrin.

Insecticide solutions were freshly prepared at a concentration of **1.33 mL/L** and applied by spraying at a dose rate of **0.05 mL/m²** to simulate environmental exposure conditions. Treatments were administered **once daily for 28 consecutive days**.

At the end of the exposure period, animals were anaesthetised with light ether vapour and humanely sacrificed. Blood samples were collected by cardiac puncture, while the brain and lung tissues were excised immediately, rinsed in ice-cold saline, and processed for biochemical and histopathological analyses.

(b) Tissue Preparation

At the end of exposure, animals were euthanised under light anaesthesia. Brain and lung tissues were excised, rinsed in ice-cold saline, blotted dry, and homogenised (10% w/v) in phosphate buffer (0.1 M, pH 7.4). The homogenates were centrifuged at 10,000 g for 15 min at 4 °C, and the supernatants were collected for biochemical assays.

2.3. Biochemical Assays

- **Reduced Glutathione (GSH):** measured using Ellman's reagent (DTNB) at 412 nm following the method described by Forman et al. [6]. Results were expressed as μM GSH/mg protein.
- **Acid Phosphatase (ACP):** determined using *p*-nitrophenyl phosphate substrate at 405 nm as outlined by Wang et al. [13].
- **Alkaline Phosphatase (ALP):** assayed with *p*-nitrophenyl phosphate buffer at pH 10.4 according to Ahmed et al. [14].
- **Protein concentrations:** determined using the Lowry method with bovine serum albumin as standard.

2.4. Histopathological Examination

Portions of brain and lung tissues were fixed in 10% neutral buffered formalin for 48 h, dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax. Sections (5 μm) were cut, mounted on slides,

and stained with haematoxylin and eosin (H&E). Slides were examined under a light microscope (Olympus CX43, Japan), and micrographs were captured for evaluation.

3. Statistical Analysis

All results were expressed as mean $\pm$ SEM ($n = 4$). Data were analysed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. Statistical significance was accepted at $p < 0.05$. Analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA).

4. Results

The attached Figure 1 presents the reduced glutathione (GSH) concentration in the brain of postpartum female Wistar albino rats exposed to various pesticide mixtures, including dichlorvos, dimethoate, and cypermethrin. Reduced glutathione, a critical antioxidant, plays a significant role in protecting brain tissues from oxidative stress caused by toxicants.

Group A (Control), which was exposed to sprayed water, exhibited the highest GSH concentration ($8.5 \pm 0.3 \mu\text{M}$), indicating a healthy, unaltered antioxidant defence system in the absence of pesticide exposure. In Group B (exposed to dichlorvos), the GSH concentration significantly decreased to $6.2 \pm 0.2 \mu\text{M}$ ($p < 0.05$) compared to the control, suggesting that dichlorvos induces oxidative stress and impairs antioxidant capacity. A further significant decline was observed in Group C (exposed to dimethoate), where the GSH level dropped to $5.9 \pm 0.2 \mu\text{M}$ ($p < 0.05$), implying that dimethoate exerts notable oxidative stress on brain tissue.

Group D (exposed to cypermethrin) demonstrated a relatively higher GSH concentration ($7.8 \pm 0.3 \mu\text{M}$) compared to Groups B and C but was still significantly lower ($p < 0.05$) than the control. This suggests that cypermethrin, while inducing oxidative stress, may have a less severe impact on brain GSH levels when administered alone.

Exposure to mixtures of pesticides resulted in more pronounced decreases in GSH levels. Group E (dichlorvos and dimethoate) showed a significant decline ($5.2 \pm 0.2 \mu\text{M}$, $p < 0.05$), indicating a potentiated oxidative effect of the combined pesticides. Similarly, Group G (dimethoate and cypermethrin) exhibited comparable GSH depletion ($5.2 \pm 0.3 \mu\text{M}$, $p > 0.05$ compared to Group E), reinforcing the compounded toxicity of pesticide mixtures.

Interestingly, Group F (dichlorvos and cypermethrin) presented a relatively higher GSH concentration ($6.9 \pm 0.2 \mu\text{M}$) compared to Groups E and G, and this difference was statistically significant ($p < 0.05$). This suggests that the dichlorvos–cypermethrin mixture may exert less oxidative stress compared to dichlorvos–dimethoate or dimethoate–cypermethrin combinations.

The most significant reduction in GSH concentration was observed in Group H (exposed to dichlorvos, dimethoate, and cypermethrin simultaneously), where GSH dropped to $4.3 \pm 0.1 \mu\text{M}$ ($p < 0.05$ compared to all other groups), indicating an intensified oxidative effect of the triple pesticide mixture.

Statistical analysis revealed significant differences ($p < 0.05$) in GSH concentrations between the control and all pesticide-exposed groups, with Group H showing the most severe depletion. Groups exposed to mixtures, such as Groups E and G, were not significantly different ($p > 0.05$) from each other but were significantly lower than the

control and some single pesticide groups. These findings emphasize the heightened oxidative stress caused by combined pesticide exposure compared to individual pesticides, highlighting the need for further investigation into their synergistic toxic effects.

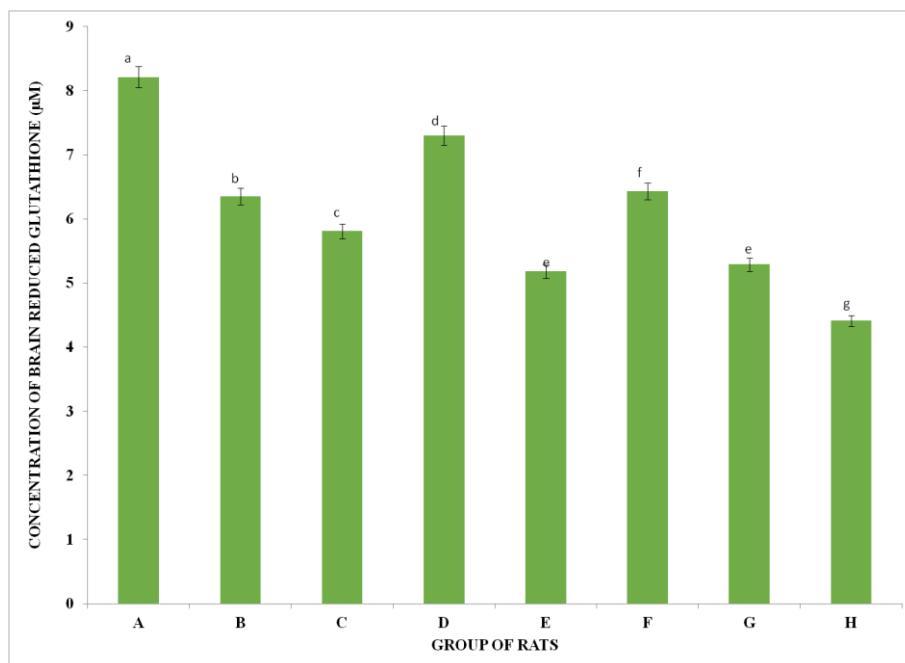


Figure 1. Reduced glutathione (GSH) concentration in the brain of postpartum female Wistar albino rats exposed to mixture of dichlorvos, dimethoate and cypermethrin. Calculated values are means of four determinations $\pm$ SEM. Bars bearing different alphabets are significantly different ($p < 0.05$).

Figure 2 shows the reduced glutathione (GSH) concentration in the lungs of postpartum female Wistar albino rats exposed to dichlorvos, dimethoate, cypermethrin, and their combinations. Reduced glutathione, a key antioxidant, helps protect lung tissues from oxidative stress and cellular damage caused by toxic substances.

Group A (Control), exposed only to sprayed water, exhibited the highest GSH concentration ($27.0 \pm 0.4 \mu\text{M}$), indicating optimal antioxidant defence and absence of oxidative stress. This group served as the baseline for comparison.

Group B (dichlorvos) and Group D (cypermethrin) both displayed significant reductions in GSH ($18.0 \pm 0.3 \mu\text{M}$ each, $p < 0.05$ vs. control), suggesting that exposure to these pesticides individually leads to oxidative stress. Their similar values indicate that dichlorvos and cypermethrin exert comparable effects on lung GSH depletion.

In Group C (dimethoate), GSH dropped further to $13.0 \pm 0.2 \mu\text{M}$ ($p < 0.05$ vs. control and Groups B/D), indicating a stronger oxidative impact compared to dichlorvos or cypermethrin alone.

Notably, Group E (dichlorvos + dimethoate) showed a further decline to $10.0 \pm 0.3 \mu\text{M}$ ($p < 0.05$), reflecting a potentiated oxidative effect when both pesticides were combined.

Group F (dichlorvos + cypermethrin) exhibited an intermediate GSH level ($15.0 \pm 0.2 \mu\text{M}$), significantly lower than the control but higher ($p < 0.05$) than Group E, indicating a less severe oxidative effect than the dichlorvos + dimethoate combination.

Group G (dimethoate + cypermethrin) recorded $18.0 \pm 0.4 \mu\text{M}$, comparable to Groups B and D ($p > 0.05$), suggesting that this combination does not exacerbate oxidative stress beyond their individual impacts.

The most significant depletion occurred in Group H (triple mixture), where GSH fell to $6.0 \pm 0.2 \mu\text{M}$ ($p < 0.05$ vs. all groups), underscoring the intensified oxidative stress and depletion of antioxidant defences caused by simultaneous exposure to all three pesticides.

The figure demonstrates that exposure to individual pesticides causes significant reductions in lung GSH concentration, with dimethoate appearing to have the most pronounced individual effect. Combined exposures, particularly dichlorvos and dimethoate (Group E) and the triple mixture (Group H), lead to further reductions, indicating amplified toxic response oxidative stress. These findings emphasize the heightened risk associated with combined pesticide exposure and the potential for severe oxidative damage in lung tissues.

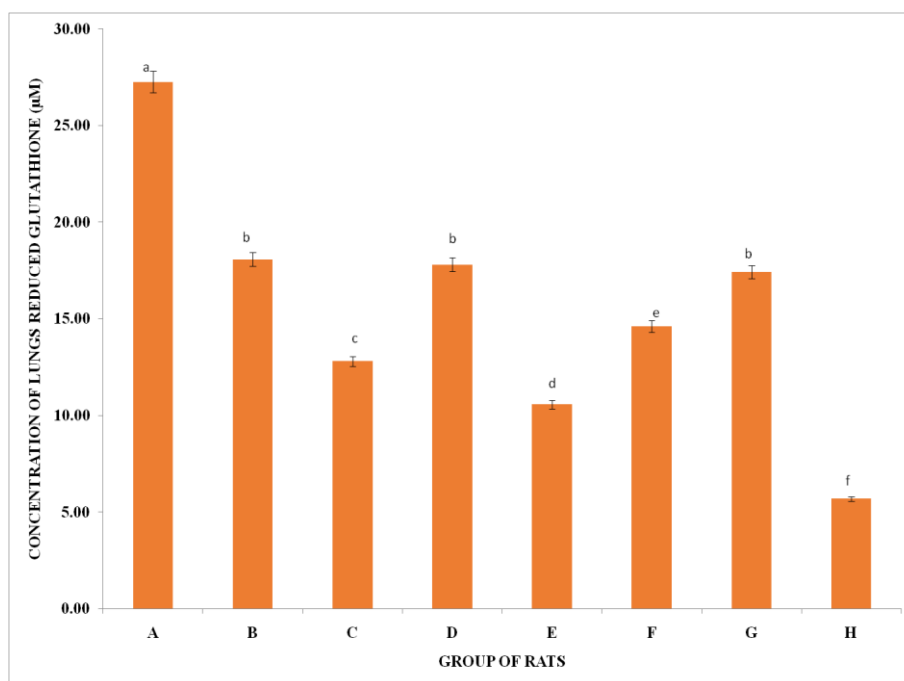


Figure 2. Reduced glutathione (GSH) concentration in the lungs of postpartum female Wistar albino rats exposed to mixture of dichlorvos, dimethoate and cypermethrin. Calculated values are means of four determinations $\pm$ SEM.

Bars bearing different alphabets are significantly different ($p < 0.05$).

Figure 3 illustrates the specific activity of acid phosphatase (ACP) in the brain of postpartum female Wistar albino rats exposed to dichlorvos, dimethoate, cypermethrin, and their combinations. Acid phosphatase, a lysosomal enzyme, is often used as a marker of cellular damage or lysosomal membrane integrity, with elevated activity indicating stress or tissue injury.

Group A (Control), which was exposed to sprayed water, exhibited the lowest ACP activity (0.03 ± 0.01 U/mg protein), reflecting the absence of cellular stress or damage under normal conditions.

Group B (dichlorvos) and Group C (dimethoate) showed significantly elevated ACP activity (0.23 ± 0.02 and 0.18 ± 0.02 U/mg protein, respectively; $p < 0.05$), indicating that both pesticides induce cellular stress in the brain, with dichlorvos exerting a stronger effect than dimethoate when administered individually.

Group D (cypermethrin) presented a relatively lower ACP activity (0.10 ± 0.01 U/mg protein), still significantly higher than the control ($p < 0.05$) but lower than Groups B and C, suggesting a less pronounced lysosomal impact when administered alone.

Exposure to pesticide mixtures produced more marked increases. Group E (dichlorvos + dimethoate) displayed 0.60 ± 0.03 U/mg protein ($p < 0.05$ vs. control and all single pesticide groups), reflecting a potentiated effect on lysosomal stress. Group F (dichlorvos + cypermethrin) showed intermediate ACP activity (0.42 ± 0.02 U/mg protein), significantly higher than single exposures but lower than Group E.

Group G (dimethoate + cypermethrin) recorded 0.52 ± 0.03 U/mg protein, not significantly different from Group F ($p > 0.05$) but significantly higher than the control and individual pesticide groups ($p < 0.05$).

The most dramatic elevation occurred in Group H (triple mixture), where ACP reached 1.20 ± 0.04 U/mg protein ($p < 0.05$ vs. all groups), underscoring the intensified lysosomal disruption and cellular stress caused by simultaneous exposure to all three pesticides.

ACP activity in the brain of postpartum rats increased significantly upon exposure to dichlorvos, dimethoate, and cypermethrin, either individually or in combination. The greatest increases were observed in groups exposed to pesticide mixtures, particularly Group E (dichlorvos and dimethoate) and Group H (triple mixture). These results suggest that combined pesticide exposure exacerbates lysosomal damage and cellular stress in brain tissues, with the triple mixture producing the most severe effects.

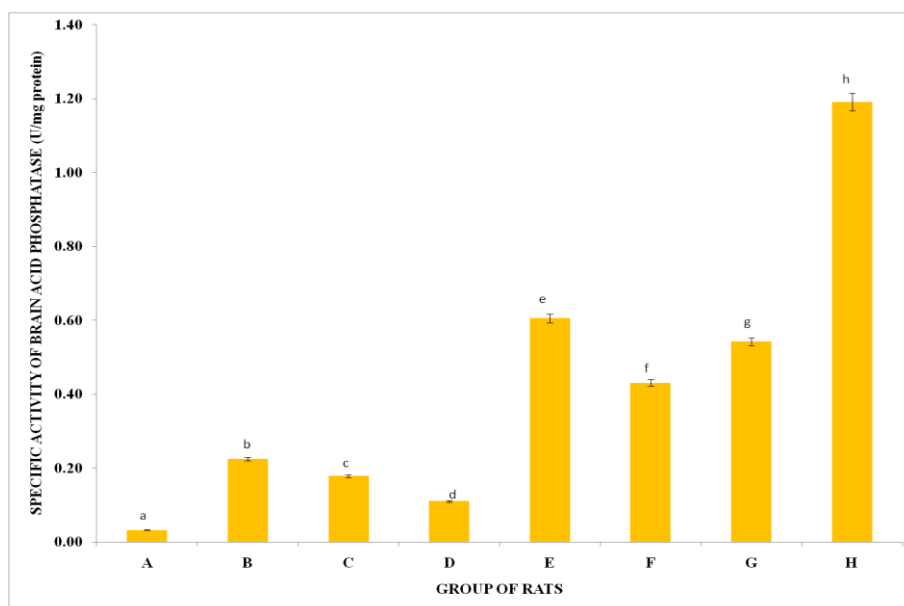


Figure 3. Specific activity of acid phosphatase (ACP) in the brain of postpartum female Wistar albino rats exposed to mixture of dichlorvos, dimethoate and cypermethrin. Calculated values are means of four determinations $\pm$ SEM.

Bars bearing different alphabets are significantly different ($p < 0.05$).

Figure 4 depicts the specific activity of acid phosphatase (ACP) in the lungs of postpartum female Wistar albino rats exposed to dichlorvos, dimethoate, cypermethrin, and their mixtures. Acid phosphatase activity serves as a marker of lysosomal membrane integrity, with elevated levels suggesting cellular stress, lysosomal leakage, or damage.

Group A (Control), which received only sprayed water, exhibited the lowest ACP activity (0.15 ± 0.01 U/mg protein), serving as the baseline for normal cellular conditions without pesticide-induced stress.

Exposure to individual pesticides led to significant increases in ACP activity. Group B (dichlorvos) and Group D (cypermethrin) both displayed 0.42 ± 0.02 U/mg protein ($p < 0.05$ vs. control), indicating moderate cellular stress. Group C (dimethoate) showed a slightly higher value (0.48 ± 0.02 U/mg protein), suggesting that dimethoate exerts a stronger oxidative and lysosomal impact than dichlorvos or cypermethrin alone.

When mixtures were administered, further elevations were observed. Group E (dichlorvos + dimethoate) recorded 0.50 ± 0.02 U/mg protein, not significantly different from Group C ($p > 0.05$) but significantly higher than the control ($p < 0.05$), reflecting cumulative stress.

Group F (dichlorvos + cypermethrin) rose sharply to 0.80 ± 0.03 U/mg protein ($p < 0.05$ vs. single exposures), indicating pronounced lysosomal disruption. Group G (dimethoate + cypermethrin) produced a similar response (0.82 ± 0.03 U/mg protein, $p > 0.05$ vs. F), confirming that this mixture also exacerbates lysosomal injury.

The highest ACP activity was observed in Group H (triple mixture), which reached 0.85 ± 0.03 U/mg protein ($p < 0.05$ vs. all groups), underscoring the intensified toxicity of combined pesticide exposure and its role in extensive cellular damage and lysosomal destabilisation in lung tissues.

ACP activity in the lungs increased significantly in response to pesticide exposure, with the greatest effects observed in groups exposed to combined pesticides, particularly the triple mixture in Group H. These findings suggest that exposure to pesticide combinations amplifies cellular stress and lysosomal damage in lung tissues, highlighting the risks of co-exposure to multiple pesticides.

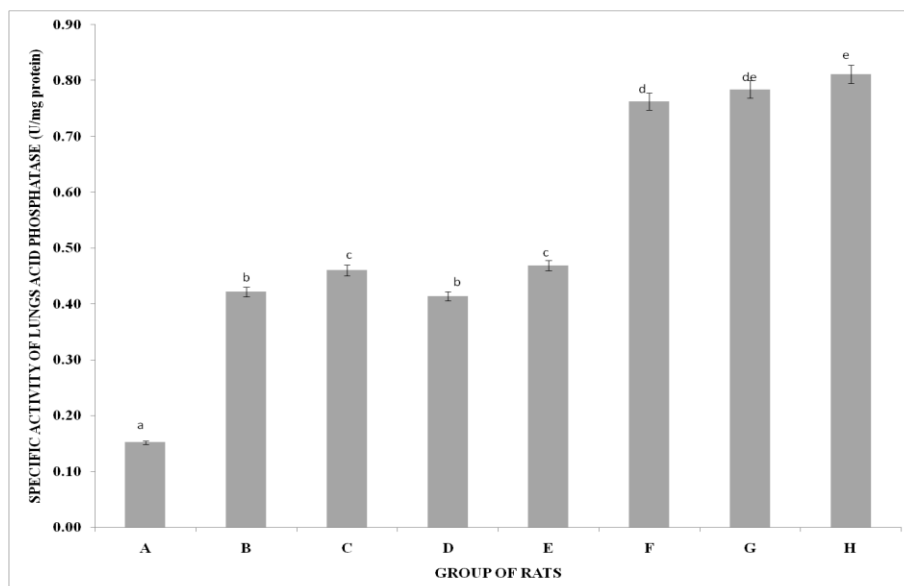


Figure 4. Specific activity of acid phosphatase (ACP) in the lungs of postpartum female Wistar albino rats exposed to mixture of dichlorvos, dimethoate and cypermethrin. Calculated values are means of four determinations $\pm$ SEM.

Bars bearing different alphabets are significantly different ($p < 0.05$).

Figure 5 shows the specific activity of alkaline phosphatase (ALP) in the brain of postpartum female Wistar albino rats exposed to dichlorvos, dimethoate, cypermethrin, and their combinations. Alkaline phosphatase is a marker

enzyme associated with membrane transport and structural integrity, with changes in its activity reflecting membrane damage, impaired cellular function, or metabolic alterations.

Group A (Control), exposed only to sprayed water, exhibited the highest ALP activity (1.25 ± 0.04 U/mg protein), representing normal brain function and membrane integrity.

Group B (dichlorvos) and Group D (cypermethrin) both showed significant reductions (0.82 ± 0.03 U/mg protein; $p < 0.05$ vs. control), indicating that each pesticide impairs membrane stability and metabolic processes. Group C (dimethoate) produced a further decrease to 0.75 ± 0.03 U/mg protein, significantly lower than Group B ($p < 0.05$), suggesting a stronger inhibitory effect of dimethoate.

Exposure to mixtures caused more pronounced inhibition. Group E (dichlorvos + dimethoate) recorded 0.42 ± 0.02 U/mg protein ($p < 0.05$ vs. single pesticide groups). Group F (dichlorvos + cypermethrin) dropped further to 0.38 ± 0.02 U/mg protein, reflecting severe disruption of brain cellular function.

Group G (dimethoate + cypermethrin) exhibited an even greater decline (0.25 ± 0.02 U/mg protein, $p < 0.05$ vs. B, C, and D). The most dramatic inhibition occurred in Group H (triple mixture), where ALP fell to 0.18 ± 0.01 U/mg protein ($p < 0.05$ vs. all groups), underscoring the intensified inhibitory impact of combined pesticide exposure on brain enzymatic activity.

The figure demonstrates a significant dose-dependent reduction in brain ALP activity following exposure to individual pesticides and their combinations. The greatest inhibitory effects were observed in groups exposed to pesticide mixtures, particularly Group H (triple mixture). These results suggest that combined pesticide exposure exacerbates membrane instability and cellular dysfunction, with potential implications for neurotoxicity and brain metabolic impairment.

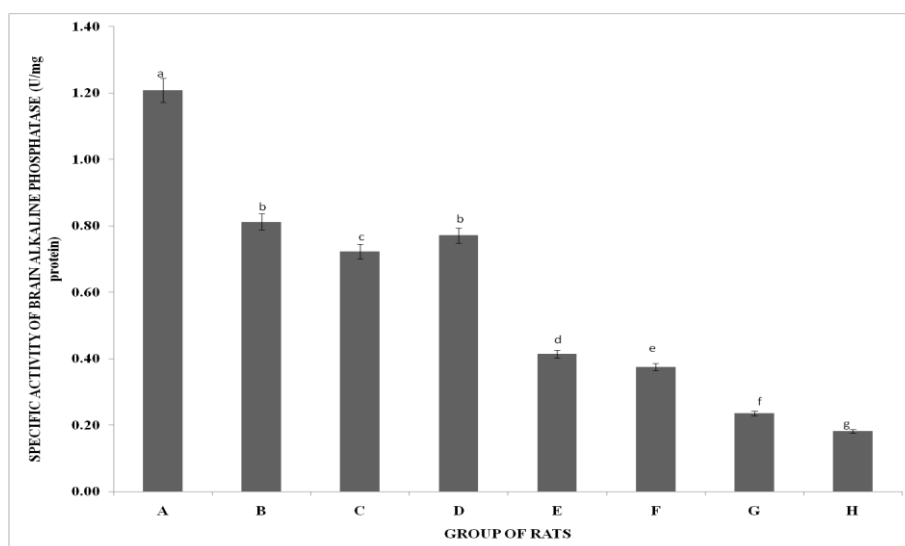


Figure 5. Specific activity of alkaline phosphatase (ALP) in the brain of postpartum female Wistar albino rats exposed to mixture of dichlorvos, dimethoate and cypermethrin. Calculated values are means of four determinations $\pm$ SEM. Bars bearing different alphabets are significantly different ($p < 0.05$).

Figure 6 illustrates the specific activity of alkaline phosphatase (ALP) in the lungs of postpartum female Wistar albino rats exposed to dichlorvos, dimethoate, cypermethrin, and their combinations. ALP is an important enzyme

involved in membrane transport and tissue integrity, and its activity reflects the degree of cellular function or disruption.

Group A (Control) and Group B (dichlorvos) exhibited the highest ALP activities (1.18 ± 0.04 and 1.15 ± 0.03 U/mg protein, respectively), with no significant difference between them ($p > 0.05$), suggesting that dichlorvos alone does not substantially alter ALP activity in lung tissue.

Group C (dimethoate) showed a significant reduction to 1.00 ± 0.03 U/mg protein ($p < 0.05$ vs. control), indicating that dimethoate disrupts lung membrane integrity or enzymatic function. Group D (cypermethrin) caused a further decrease to 0.85 ± 0.03 U/mg protein ($p < 0.05$ vs. A and B), highlighting a stronger inhibitory effect than dichlorvos. Combined exposures produced more pronounced effects. Group E (dichlorvos + dimethoate) dropped to 0.78 ± 0.02 U/mg protein ($p < 0.05$ vs. control and single pesticides), while Group F (dichlorvos + cypermethrin) fell further to 0.62 ± 0.02 U/mg protein, reflecting more severe disruption of lung cellular processes.

Group G (dimethoate + cypermethrin) showed 0.65 ± 0.02 U/mg protein, slightly higher than Group F but still significantly lower than Groups A–D ($p < 0.05$). The most substantial inhibition was observed in Group H (triple mixture), which recorded 0.48 ± 0.02 U/mg protein ($p < 0.05$ vs. all groups), underscoring the exacerbated inhibitory effects of combined pesticide exposure on lung ALP activity.

Exposure to dichlorvos alone had minimal effects on lung ALP activity, while dimethoate and cypermethrin individually caused significant reductions. Combined exposures, particularly in Groups E, F, and G, led to more pronounced inhibition, with the triple mixture in **Group H** producing the most severe effects. These results highlight the synergistic impact of pesticide combinations, which exacerbate lung cellular dysfunction and compromise membrane stability.

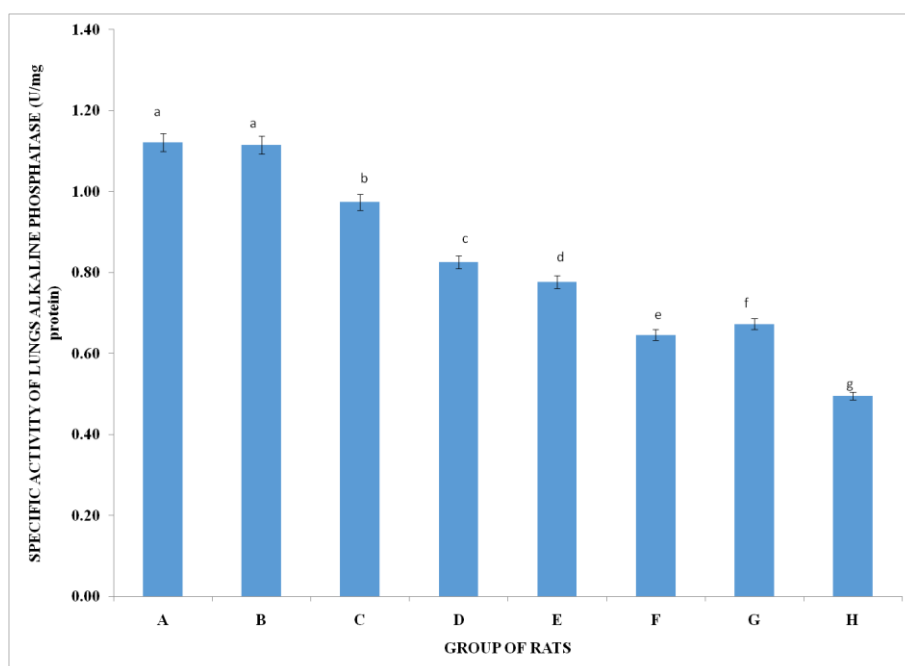


Figure 6. Specific activity of alkaline phosphatase (ALP) in the lungs of postpartum female Wistar albino rats exposed to mixture of dichlorvos, dimethoate and cypermethrin. Calculated values are means of four determinations $\pm$ SEM. Bars bearing different alphabets are significantly different ($p < 0.05$).

The histopathological findings in **Plates 1 to 8** highlight the effects of dichlorvos, dimethoate, cypermethrin, and their combinations on the brain tissues of postpartum female Wistar albino rats. Each group presents distinct alterations, ranging from normal brain architecture to severe cellular damage, depending on the type and combination of pesticide exposure.

In **Group A (Control, Plate 1)**, the micrograph reveals normal brain architecture. The brain tissue comprises large neurons (LN), small neurons (SM), gliocytes (GL), and a well-organized axion fibre (AF) meshwork, indicating no observable pathology or cellular damage. This group serves as the baseline for comparison, reflecting the healthy condition of brain tissues without pesticide exposure.

For **Group B (Dichlorvos, Plate 2)**, the brain micrograph shows multiform layer nuclear pyknosis (NP) and granular cell clumping (GC). Nuclear pyknosis, a sign of irreversible neuronal injury, indicates cellular stress and damage, likely due to oxidative stress caused by dichlorvos. Granular cell clumping further reflects neuronal dysfunction, suggesting significant neurotoxicity from dichlorvos exposure.

In **Group C (Dimethoate, Plate 3)**, the findings include an oedematous granular layer (OG) and granular cell nuclear pyknosis (NP). The presence of oedema suggests fluid accumulation, which disrupts brain tissue integrity and function. Combined with nuclear pyknosis, these changes point to dimethoate's ability to induce cellular stress, inflammation, and neurodegeneration.

The micrograph for **Group D (Cypermethrin, Plate 4)** displays glial cell degeneration (GD) and nuclear pyknosis (NP). Glial cell degeneration indicates damage to supportive brain cells, which play a critical role in maintaining neuronal health. The presence of nuclear pyknosis further suggests cypermethrin-induced neurotoxicity, compromising both glial and neuronal cell integrity.

In **Group E (Dichlorvos and Dimethoate, Plate 5)**, granular layer oedema (GO) is observed. This oedema reflects a synergistic toxic effect of dichlorvos and dimethoate, causing more severe fluid accumulation in brain tissues compared to individual exposures. Such oedematous changes impair cellular function and highlight the combined impact of these pesticides.

For **Group F (Dichlorvos and Cypermethrin, Plate 6)**, cerebral vascular congestion (VC) is evident. Vascular congestion reflects impaired blood flow and possible inflammation, suggesting that the combination of dichlorvos and cypermethrin affects both vascular and neuronal integrity. This further supports the hypothesis of a cumulative toxic effect from pesticide combinations.

Interestingly, **Group G (Dimethoate and Cypermethrin, Plate 7)** shows relatively normal brain architecture, including intact polymorphic (PO), pyramidal (PY), and granular cell layers (GC). The absence of severe histopathological changes suggests that the combination of dimethoate and cypermethrin may exhibit less toxicity compared to other pesticide mixtures, although subtle damage cannot be ruled out.

The most severe damage is observed in **Group H (Dichlorvos, Dimethoate, and Cypermethrin, Plate 4.8)**, where pyramidal cell degeneration (PD) and multiform cell degeneration (MD) are prominent. These findings indicate extensive neuronal injury and structural disruption, reflecting an amplified toxic response of the three pesticides.

Such degeneration compromises brain tissue integrity, suggesting a heightened neurotoxic risk with triple pesticide exposure.

In summary, individual pesticide exposures caused distinct forms of brain damage, such as nuclear pyknosis, oedema, and glial cell degeneration. Combined exposures, particularly dichlorvos and dimethoate (Group E) and the triple pesticide mixture (Group H), led to exacerbated pathological changes, including oedema, vascular congestion, and widespread cellular degeneration. The findings underscore the neurotoxic potential of pesticide combinations, with the triple exposure showing the most severe effects. These results highlight the importance of assessing the cumulative impacts of pesticide mixtures on brain health.

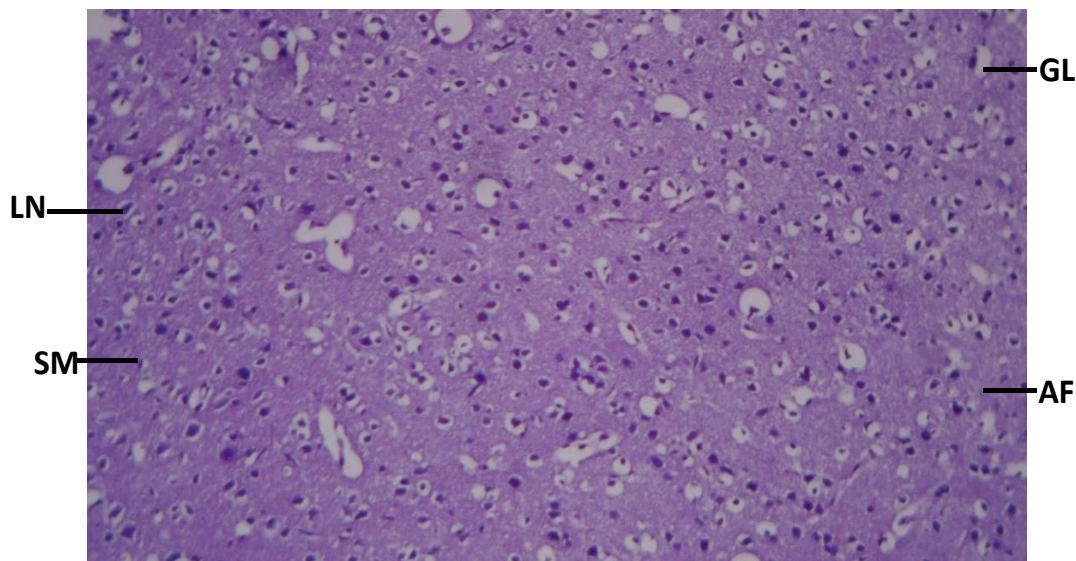


Plate 1. Group A: Histopathology micrograph of brain of postpartum female Wistar albino rats exposed to sprayed water showing: admixture of neuron cell bodies of large neurons (LN), small neurons (SM) supported by gliocytes (GL) all surrounded by a meshwork of axionfibres (AF): H&E 100 X.

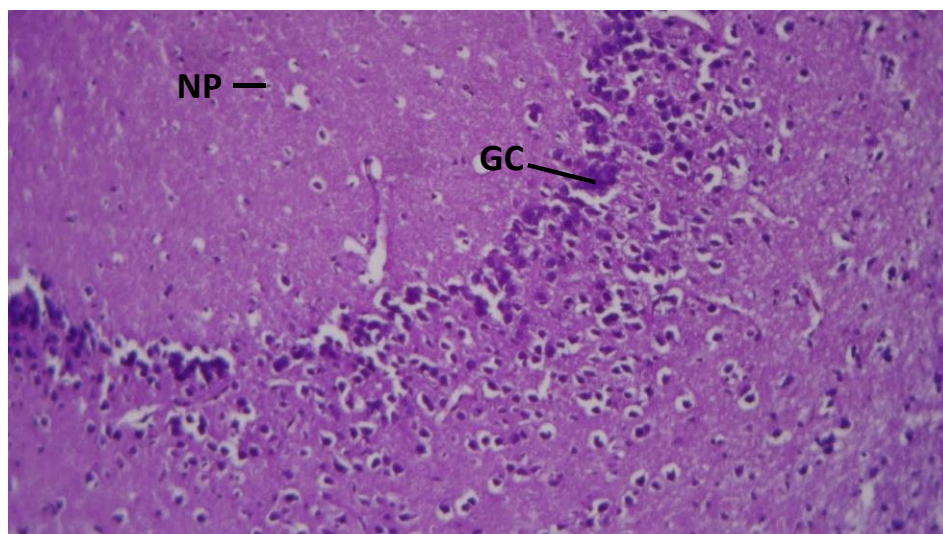


Plate 2. Group B: Histopathology micrograph of brain of postpartum female Wistar albino rats exposed to dichlorvos showing: multiform layer nuclear pyknosis (NP), granular cell clumping (GC) in the granular layer: H&E 100 X.

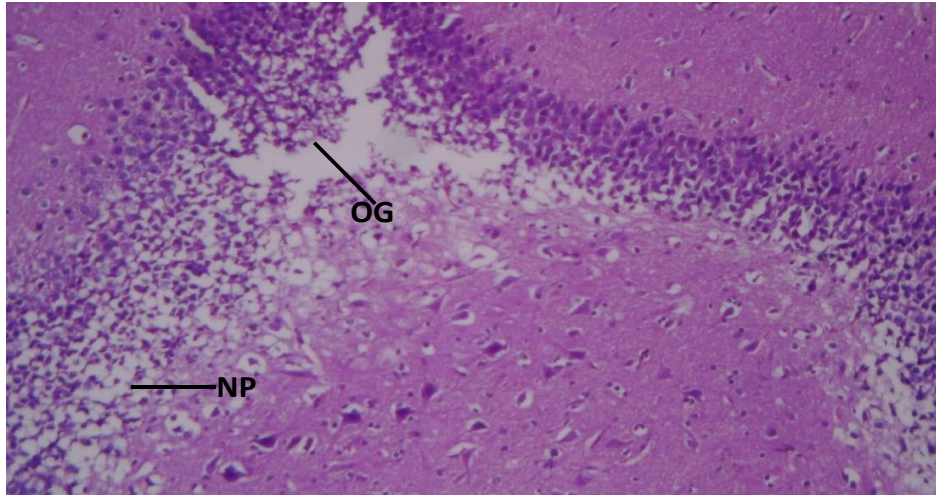


Plate 3. Group C: Histopathology micrograph of brain of postpartum female Wistar albino rats exposed to dimethoate showing: oedematous granular layer (OG) and granular cell nuclear pyknosis (NP): H&E 100 X.

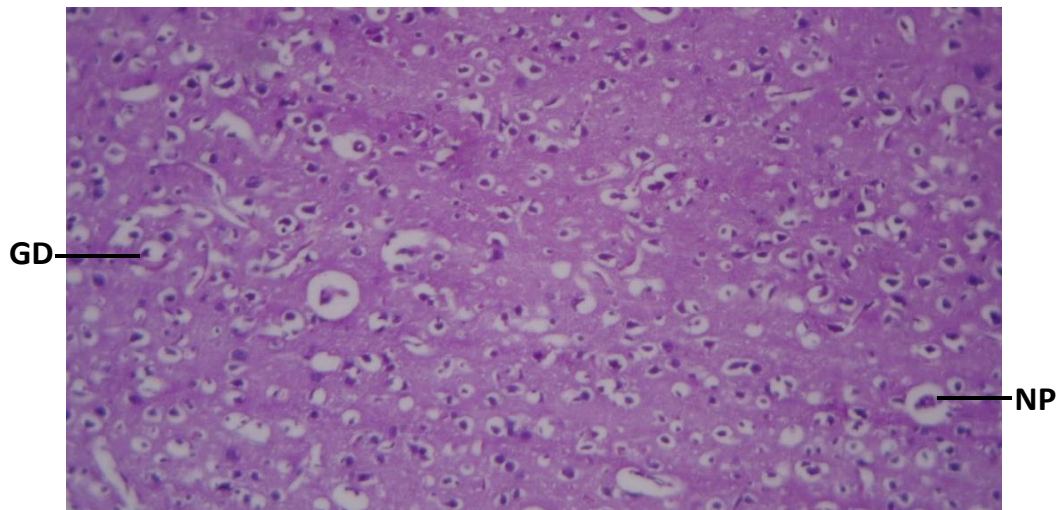


Plate 4. Group D: Histopathology micrograph of brain of postpartum female Wistar albino rats exposed to cypermethrin showing: glial cell degeneration (GD) and normal nuclear pyknosis (NP): H&E 100 X.

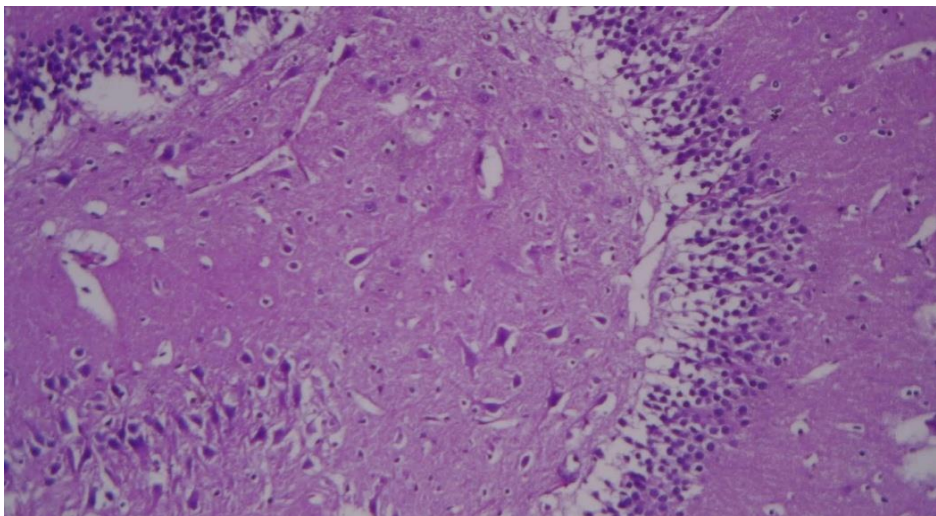


Plate 5. Group E: Histopathology micrograph of brain of postpartum female Wistar albino rats exposed to dichlorvos and dimethoate showing: granular layer oedema (GO): H&E 100 X.

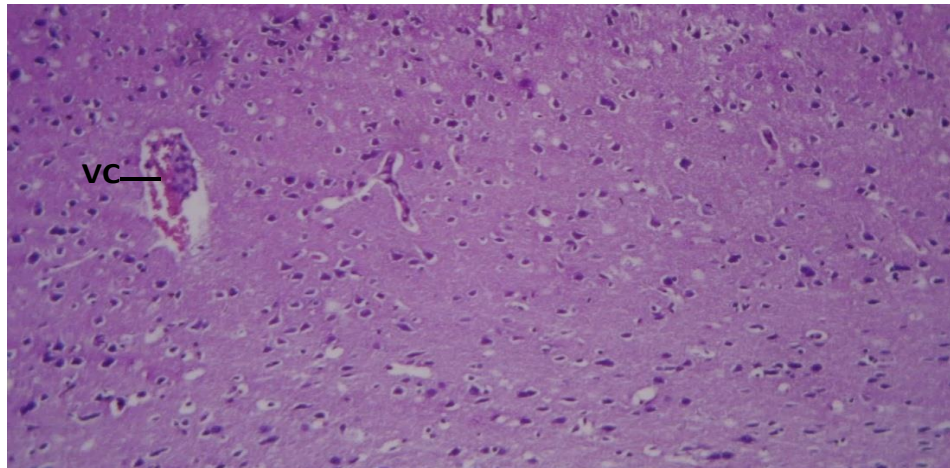


Plate 6. Group F: Histopathology micrograph of brain of postpartum female Wistar albino rats exposed to dichlorvos and cypermethrin showing cerebral vascular congestion (VC): H&E 100 X.

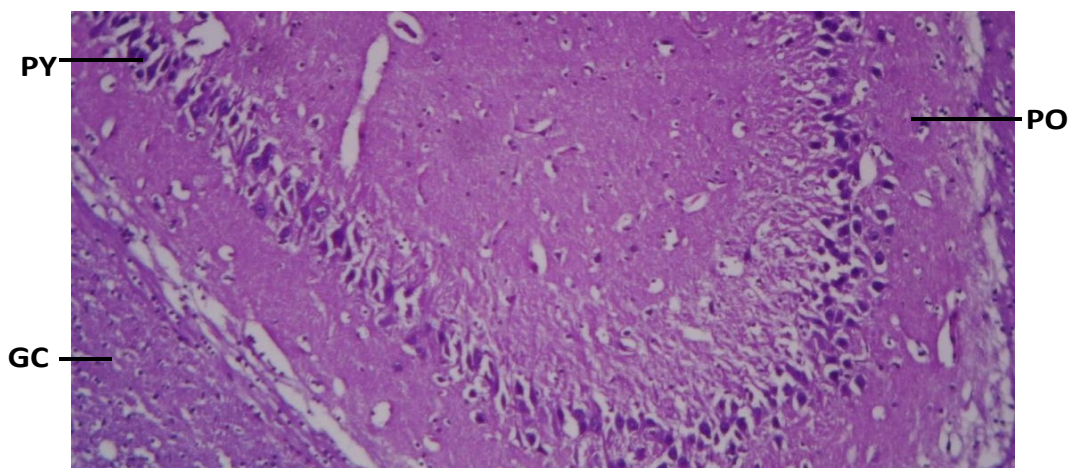


Plate 7. Group G: Histopathology micrograph of brain of postpartum female Wistar albino rats exposed to dimethoate and cypermethrin showing normal polymorphic (PO), pyramidal (PY) and granular cell layers (GC): H&E 100 X.

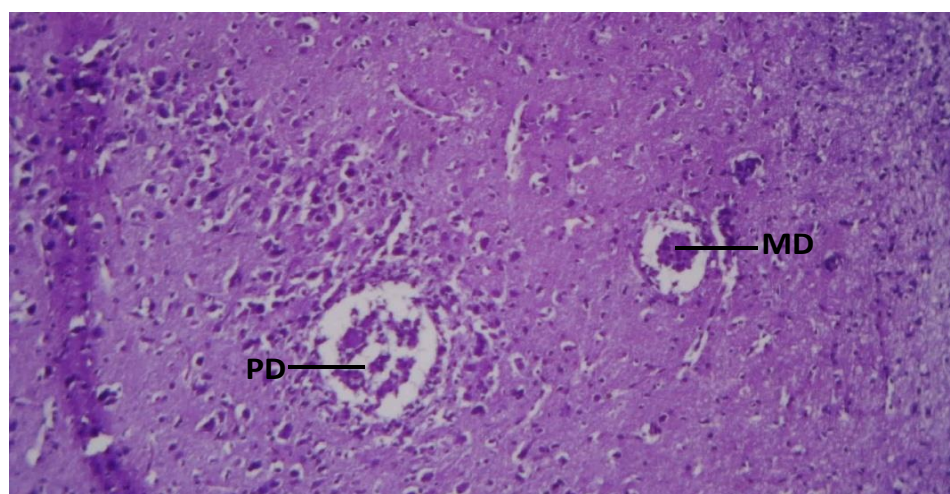


Plate 8. Group H: Histopathology micrograph of brain of postpartum female Wistar albino rats exposed to dichlorvos, dimethoate, and cypermethrin showing pyramidal cell degeneration (PD) and multiform cell degeneration (MD): H&E x 100 X.

The histopathological findings described in **Plates 9 to 16** detail the effects of dichlorvos, dimethoate, cypermethrin, and their combinations on the lungs of postpartum female Wistar albino rats. The results reflect varying degrees of structural damage, inflammatory responses, and cellular necrosis depending on the type and combination of pesticide exposure.

In **Group A (Control, Plate 9)**, the micrograph shows normal lung architecture with well-defined **alveolar spaces (AS)**, **terminal bronchioles (TE)**, and minimal **perivascular interstitial infiltrates of inflammatory cells (PI)**. This indicates healthy lung tissues and serves as the baseline for comparison with pesticide-exposed groups.

For **Group B (Dichlorvos, Plate 10)**, the lungs show **vascular hypertrophy (VH)** and **bronchiolar mucosal necrosis (BN)**. Vascular hypertrophy reflects thickening of vascular walls, which can impair pulmonary circulation. Bronchiolar necrosis indicates cellular death, highlighting the toxic effects of dichlorvos on the respiratory epithelium.

In **Group C (Dimethoate, Plate 11)**, severe pathological changes are observed, including **atelectasis (AT)** and **interstitial exudates of inflammation (EI)**. Atelectasis, which is the collapse of alveoli, disrupts normal gas exchange, while inflammatory exudates suggest an immune response to tissue damage. These findings demonstrate the severe impact of dimethoate on pulmonary function and integrity.

The micrograph for **Group D (Cypermethrin, Plate 12)** reveals **inflammatory exudates (IE)**, **thick bronchiolar secretion (BS)**, and **bronchiolar ulceration (BU)**. The presence of ulceration and exudates indicates significant injury to the bronchiolar lining, likely caused by cypermethrin-induced irritation and inflammation. Thick secretions further suggest compromised pulmonary clearance mechanisms.

For **Group E (Dichlorvos and Dimethoate, Plate 13)**, the lungs show a combination of **normal alveoli (AL)**, **inflammatory exudates (IE)**, and **bronchiolar ulceration (BU)**. While some areas of the lung appear intact, the presence of inflammatory exudates and ulceration suggests that the combination of dichlorvos and dimethoate exacerbates tissue injury compared to individual exposures.

In **Group F (Dichlorvos and Cypermethrin, Plate 14)**, the micrograph shows **bronchiolar ulceration (BU)** and **normal alveoli (AL)**. Although the alveoli appear relatively preserved, bronchiolar ulceration indicates significant toxicity to the airways caused by the combined effects of dichlorvos and cypermethrin.

Interestingly, **Group G (Dimethoate and Cypermethrin, Plate 15)** shows largely normal lung architecture, including **normal alveoli (AL)**, **terminal bronchiole (TB)**, and **bronchial artery (BA)**. The absence of severe histopathological changes suggests that the combination of dimethoate and cypermethrin may not induce as much pulmonary damage as other combinations, though subtle effects cannot be ruled out.

The most severe lung damage is observed in **Group H (Dichlorvos, Dimethoate, and Cypermethrin, Plate 16)**. The micrograph shows extensive **interstitial exudates (IE)** and **bronchiolar necrosis (BN)**. Interstitial exudates reflect severe inflammation, while bronchiolar necrosis indicates widespread tissue death. These findings suggest a exacerbated effects of the three pesticides, resulting in significant structural and functional compromise of the lungs.

4.1. Overall Interpretation and Conclusion

The histopathological analysis shows that exposure to individual pesticides (dichlorvos, dimethoate, and cypermethrin) leads to varying degrees of lung injury, including vascular hypertrophy, atelectasis, inflammatory exudates, and bronchiolar ulceration. Combined pesticide exposures, particularly **Group E** (dichlorvos and dimethoate) and **Group H** (triple mixture), exacerbate these effects, leading to more severe tissue damage, inflammation, and necrosis. Notably, **Group G** (dimethoate and cypermethrin) displayed relatively preserved lung architecture, suggesting reduced toxicity compared to other combinations.

The findings emphasize the heightened risk of combined pesticide exposure, which significantly compromises pulmonary structure and function. This highlights the need for stricter regulation and monitoring of pesticide mixtures to minimize adverse health impacts on respiratory tissues.

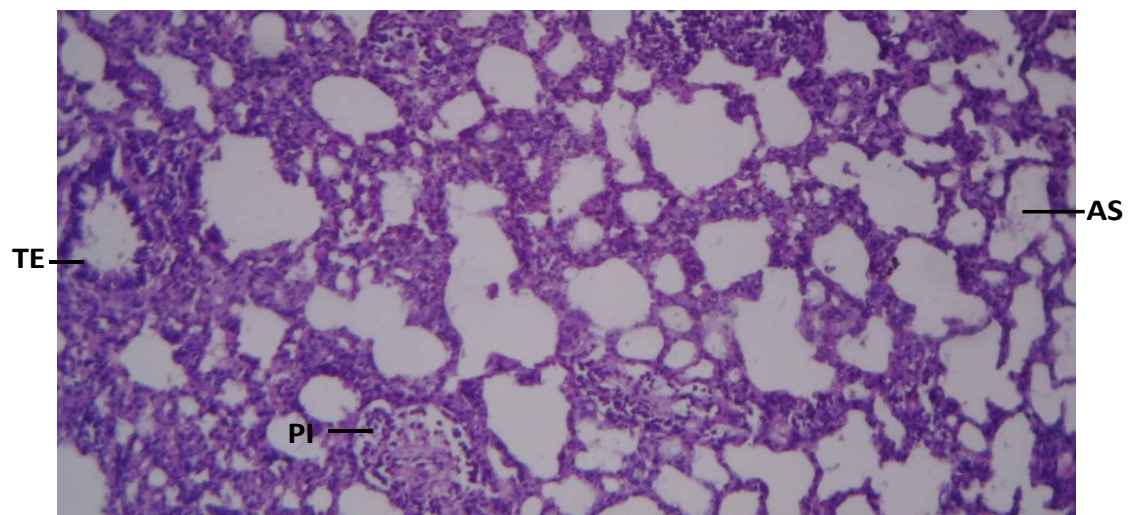


Plate 9. Group A: Histopathology micrograph of lungs of postpartum female Wistar albino rats exposed to sprayed water showing: alveolar spaces (AS), terminal bronchiole (TE), perivascular interstitial infiltrates of inflammatory cells (PI): H&E 100 X.

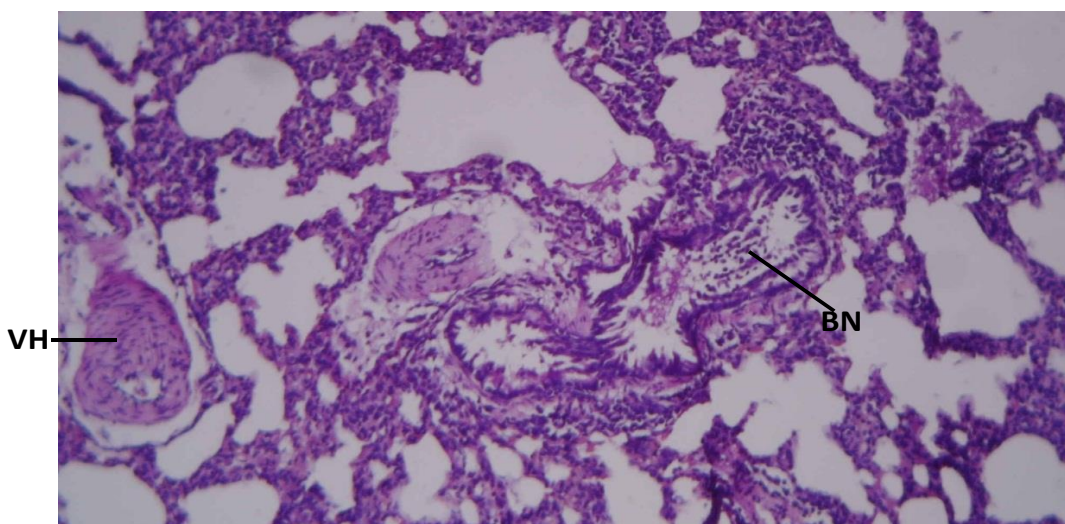


Plate 10. Group B: Histopathology micrograph of lungs of postpartum female Wistar albino rats exposed to dichlorvos showing: vascular hypertrophy (VH), bronchiolar mucosal necrosis (BN): H&E 100 X.

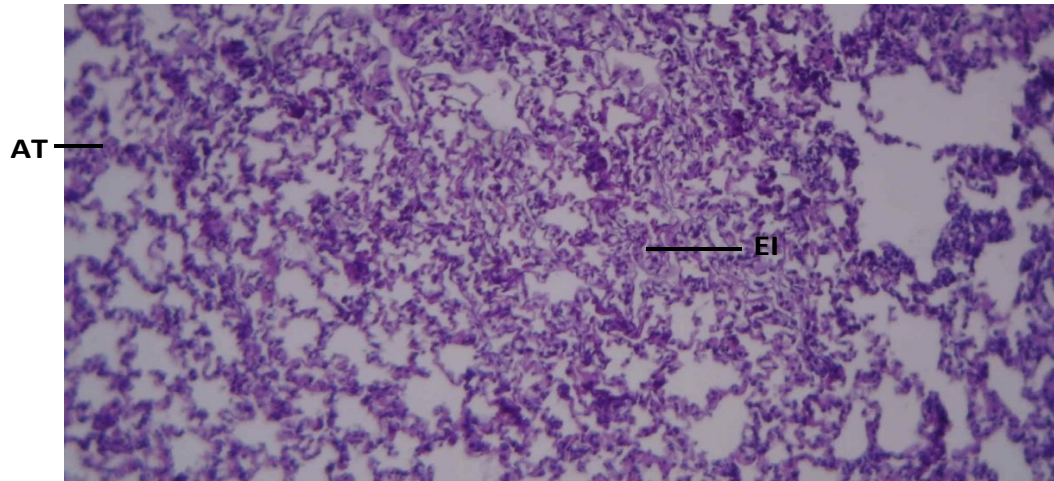


Plate 11. Group C: Histopathology micrograph of lungs of postpartum female Wistar albino rats exposed to dimethoate showing: severe atelectasis (AT) and interstitial exudates of inflammation (EI): H&E 100 X.

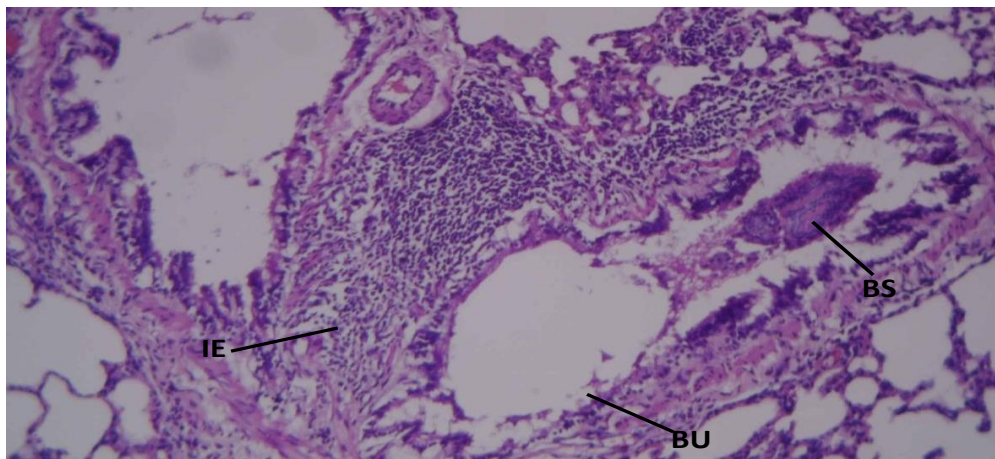


Plate 12. Group D: Histopathology micrograph of lungs of postpartum female Wistar albino rats exposed to cypermethrin showing: inflammatory exudates (IE), thick bronchiolar secretion (BS) and ulceration (BU): H&E 100 X.

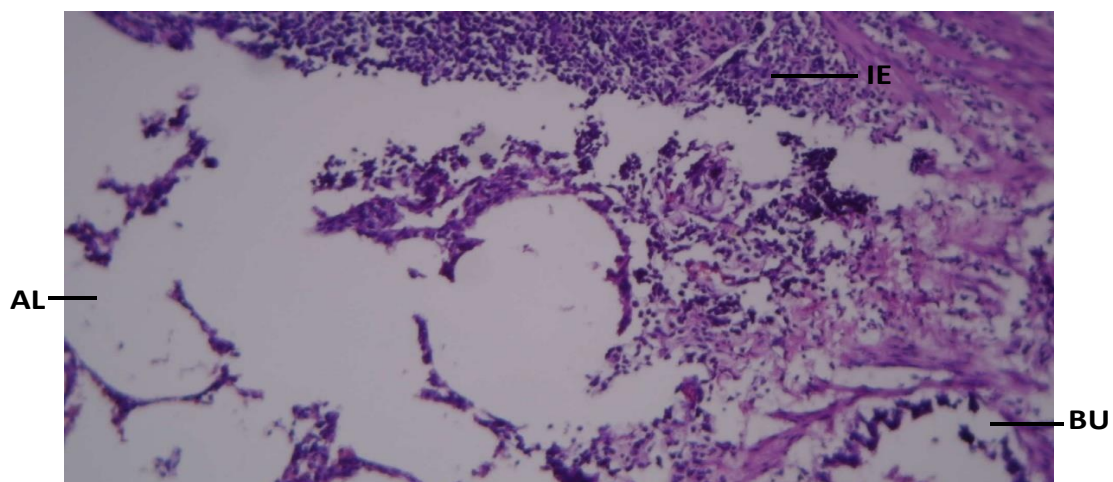


Plate 13. Group E: Histopathology micrograph of lungs of postpartum female Wistar albino rats exposed to dichlorvos and dimethoate showing normal alveoli (AL), inflammatory exudates (IE) and bronchiolar ulceration (BU): H&E 100 X.

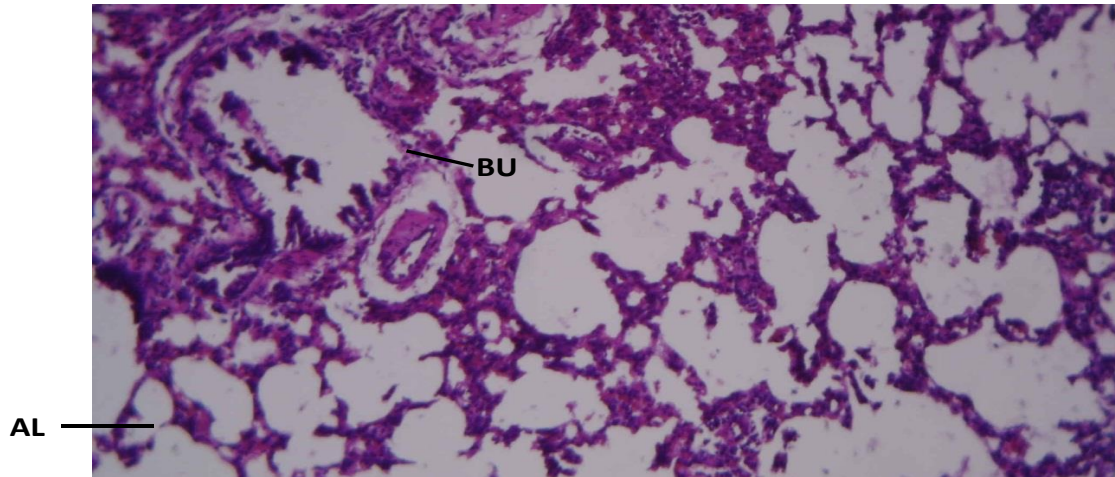


Plate 14. Group F: Histopathology micrograph of lungs of postpartum female Wistar albino rats exposed to dichlorvos and cypermethrin showing bronchiolar ulceration (BU), normal Alveoli(AL); H&E 100 X.



Plate 15. Group G: Histopathology micrograph of lungs of postpartum female Wistar albino rats exposed to dimethoate and cypermethrin showing normal alveoli (AL), terminal bronchiole (TB) and bronchial artery (BA); H&E 100 X.

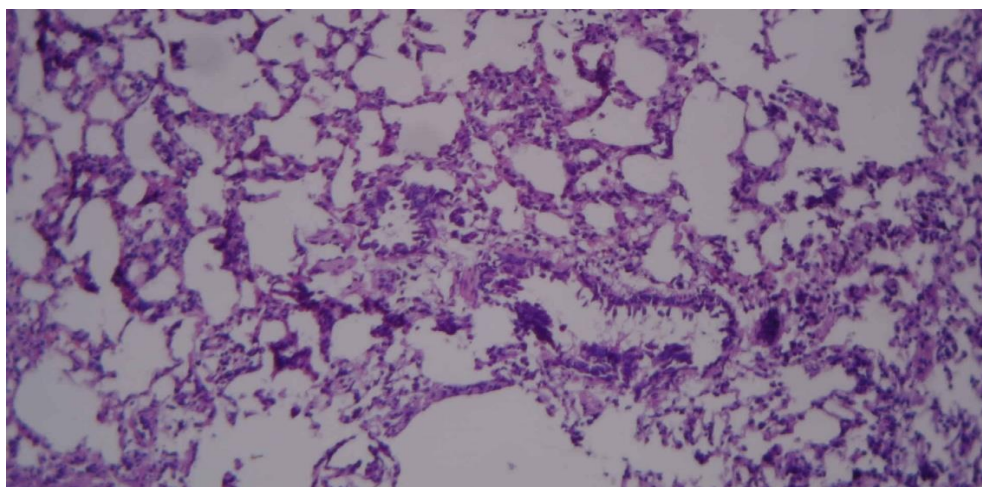


Plate 16. Group H: Histopathology micrograph of lungs of postpartum female Wistar albino rats exposed to dichlorvos, dimethoate, and cypermethrin showing interstitial exudates (IE) and bronchiolar necrosis (BN); H&E 100 X.

5. Discussion

The results of this study as provided in Figures 1 and 2 underscore a critical and tissue-specific depletion of reduced glutathione (GSH) in the brain and lungs of postpartum female Wistar albino rats following exposure to individual and combined pesticide formulations, including dichlorvos, dimethoate, and cypermethrin. GSH, as a central non-enzymatic antioxidant, plays a pivotal role in cellular detoxification and redox regulation in both neural and pulmonary tissues [6,4].

The observed patterns of GSH depletion across both organs point to a systemic oxidative burden exerted by pesticide exposure, with the lungs exhibiting generally higher baseline GSH levels but also experiencing substantial oxidative perturbations upon treatment. In both brain and lung tissues, dichlorvos and cypermethrin individually induced significant reductions in GSH levels, with dimethoate producing the most pronounced GSH depletion among single-agent exposures. These results corroborate earlier findings suggesting that dimethoate exerts greater oxidative stress, likely due to its capacity to generate reactive oxygen species (ROS) via mitochondrial disruption and cytochrome P450 activation [3,5].

The lungs, owing to their extensive vascularization and direct interface with environmental toxicants, may absorb and metabolize xenobiotics more readily, rendering them particularly susceptible to oxidative insult [15,2]. The additive or synergistic toxicities evidenced in groups co-exposed to pesticide mixtures (Groups E–H) in both organs further substantiate the notion of potentiated oxidative stress under combined exposure conditions. Specifically, the dichlorvos-dimethoate mixture (Group E) consistently demonstrated profound GSH depletion in both brain (~5.2 μM) and lung (~10 μM) tissues, supporting the hypothesis of synergistic neuro- and pulmonotoxic effects. Similar interactions have been reported in pesticide mixture studies, where co-administration leads to amplified ROS production and exhaustion of antioxidant defenses, ultimately precipitating lipid peroxidation, DNA fragmentation, and cellular apoptosis [9,10].

Notably, the triple mixture group (Group H) exhibited the most severe GSH depletion in both brain (~4.3 μM) and lung (~6 μM), suggesting a cumulative or possibly super-additive toxic response. This aligns with prior evidence that chronic or simultaneous exposure to multiple classes of pesticides—especially organophosphates and pyrethroids—can overwhelm endogenous antioxidant systems and lead to irreversible cellular damage [1,12]. Importantly, the exacerbated oxidative response in the postpartum physiological state—characterized by metabolic and hormonal shifts—could amplify vulnerability to environmental toxicants [16,13].

Furthermore, the slightly less severe depletion observed in dichlorvos-cypermethrin co-exposed groups (Group F) suggests potential antagonistic interactions or differential detoxification dynamics, as previously observed in multi-pesticide exposure studies [17,18]. However, even in these cases, GSH levels remained significantly lower than controls, affirming that any combined exposure remains a substantial oxidative threat. These findings collectively highlight the broader systemic impact of pesticide exposure, where the oxidative damage is not limited to a single organ but manifests across critical tissues, including the central nervous and respiratory systems. The implications are particularly grave for sensitive populations such as postpartum females, for whom antioxidant depletion could impair recovery and predispose to neurodegenerative or respiratory complications [11,12].

Figures 3 and 4 reveal a consistent and pronounced elevation in acid phosphatase (ACP) activity in both the brain and lungs of postpartum female Wistar albino rats exposed to dichlorvos, dimethoate, cypermethrin, and their combinations. As a lysosomal marker enzyme, ACP is released during lysosomal membrane destabilization, serving as a biochemical indicator of cellular stress, inflammation, and toxic injury [13,19]. The observed increase in ACP activity across tissues reflects pesticide-induced lysosomal damage, likely stemming from oxidative stress and membrane lipid peroxidation—a pattern well-documented in toxicological studies of organophosphates and pyrethroids.

In both organs, dichlorvos and dimethoate significantly elevated ACP activity, with dichlorvos causing the most pronounced increase in the brain and dimethoate producing higher activity in the lungs. These findings align with established mechanistic pathways through which organophosphates disrupt lysosomal function, including calcium dyshomeostasis, mitochondrial injury, and ROS generation [3,1]. Dimethoate, in particular, has been reported to induce lysosomal enzyme leakage in pulmonary cells, reflecting its strong affinity for oxidative induction in non-neuronal tissues [5,4].

The neurotoxic effects of cypermethrin, though generally milder than those of organophosphates, were also evident in this study, as shown by elevated ACP levels in both brain and lung tissues. This observation is supported by findings from earlier studies demonstrating that pyrethroids interfere with lysosomal integrity via alterations in membrane permeability and increased lipid peroxidation [8,7]. The intermediate response of ACP in the cypermethrin-exposed groups suggests a moderate but distinct potential for inducing sub-lethal cellular stress.

More compellingly, the most significant elevations in ACP activity were consistently observed in groups exposed to pesticide mixtures, particularly in Groups E through H. The combination of dichlorvos and dimethoate (Group E) produced a synergistic effect in brain tissues, with ACP activity doubling that of single pesticide groups—suggesting amplified lysosomal destabilization when both organophosphates are co-administered. This synergism may arise from compounded inhibition of acetylcholinesterase, combined ROS generation, and saturation of detoxification pathways [9,10].

In the lungs, however, the highest levels of ACP activity were observed in Groups F, G, and H—where cypermethrin was included in the mixtures—suggesting a differential organ sensitivity, possibly due to tissue-specific metabolism or variation in bioaccumulation rates [15,17]. Group H (triple mixture) presented the highest ACP activity in both tissues, pointing to a cumulative or super-additive toxic response.

Such findings emphasize that combined pesticide exposure, even at individually non-lethal doses, can precipitate overwhelming lysosomal stress and irreversible cellular injury. These results resonate with earlier studies where multiple pesticide exposures led to widespread lysosomal swelling, autophagic dysfunction, and apoptosis [18,2]. Importantly, the susceptibility of postpartum physiology to xenobiotic stress must be emphasized. The postpartum state is marked by hormonal shifts, immune modulation, and increased metabolic demand—all of which can potentiate oxidative and lysosomal vulnerabilities [11,12]. The additive impact of pesticide mixtures in this context raises significant public health concerns, particularly for agricultural workers, lactating mothers, and populations in environments with high pesticide burdens.

Moreover, Figures 5 and 6 reveals a distinct and organ-specific pattern of inhibition of alkaline phosphatase (ALP) activity in both the brain and lungs of postpartum female Wistar albino rats following exposure to dichlorvos, dimethoate, cypermethrin, and their combinations. ALP is a ubiquitous membrane-bound enzyme essential for maintaining transmembrane ion transport, neuronal metabolism, and cellular homeostasis [14,20]. Decreases in its activity are often reflective of membrane dysfunction, impaired metabolic regulation, or pesticide-induced structural damage to cells.

In brain tissues, all pesticide treatments, whether individual or combined, significantly reduced ALP activity relative to the control, with the degree of inhibition progressively increasing from single to multiple pesticide exposures. Notably, dimethoate (Group C) showed a more substantial inhibitory effect than either dichlorvos or cypermethrin alone, suggesting a greater neurotoxic potential in terms of metabolic suppression and membrane destabilization. These findings align with reports that dimethoate, via oxidative stress pathways and interference with ATP synthesis, disrupts membrane-bound enzymatic systems, including phosphatases [3,4].

The decline in ALP activity was most profound in groups exposed to pesticide mixtures. Groups E, F, and G demonstrated markedly suppressed enzymatic activity, indicating potential synergistic toxicity. The triple mixture group (Group H) exhibited the most dramatic reduction, with brain ALP activity falling to a fraction of the control value. Such additive toxicity likely results from simultaneous disruptions in multiple metabolic pathways, compounded oxidative damage, and impaired phospholipid turnover, which compromise the structural and functional integrity of neuronal membranes [1,9].

The pattern in lung tissues was somewhat distinct. While dichlorvos (Group B) had little to no effect on ALP activity, dimethoate (Group C) and cypermethrin (Group D) caused statistically significant reductions, indicating their differential affinity or metabolism in pulmonary tissue. This organ-specific variation could be attributed to differences in detoxification enzyme expression, such as cytochrome P450 isozymes, or in the local distribution of pesticide residues [2,15]. More critically, the combination treatments in lung tissue exhibited enhanced toxicity similar to the brain, with ALP activity falling progressively in Groups E through H. Group H again recorded the lowest ALP activity, affirming the hypothesis of cumulative toxicity when multiple pesticides are co-administered. The impairment of ALP in lung tissue suggests that these mixtures may compromise epithelial transport, pulmonary surfactant regulation, and alveolar barrier function—processes critical to respiratory health [19,13].

Taken together, the dual-organ response to pesticide exposure provides compelling evidence of the inhibitory impact of these xenobiotics on ALP activity. The consistent downward trend in enzymatic activity from single to combined exposures demonstrates a dose-dependent or load-dependent toxicological effect, which is especially concerning in the context of environmental and occupational co-exposure scenarios. In both the brain and lungs, Group H stands out as the most severely impacted, highlighting the significant risk posed by combined pesticide use—a scenario common in agricultural practice. The vulnerability of postpartum physiology further amplifies the concern, as this period is associated with heightened neuroendocrine plasticity, altered immunoregulation, and increased metabolic demand. These physiological changes may render the brain and lung tissues more susceptible to pesticide-induced perturbations in enzymatic activity and membrane function [12,21].

These findings highlight the need for stricter regulatory frameworks concerning pesticide mixtures and call for more research into protective strategies—such as antioxidant supplementation or alternative pest management practices—to mitigate such enzymatic and structural damage. Future studies could explore the reversibility of these inhibitory effects, investigate tissue-specific oxidative biomarkers, and employ histopathological validation to further confirm biochemical evidence.

The histopathological findings presented in Plates 1 through 8 provide compelling microscopic evidence of the neurotoxic impact of dichlorvos, dimethoate, cypermethrin, and their combinations on the brain tissue of postpartum female Wistar albino rats. The qualitative cellular alterations observed across treatment groups offer critical validation of the biochemical and enzymatic disruptions discussed in earlier figures (Figures 1–6), reinforcing the notion that pesticide exposure, particularly in mixtures, precipitates a spectrum of neuropathological changes ranging from mild alterations to profound neuronal degeneration.

The control group (Group A, Plate 1) established a histological baseline, with intact large and small neurons (LN, SM), gliocytes (GL), and a clearly delineated axion fibre (AF) network. This architecture reflects a stable neuroenvironment, free from oxidative or inflammatory insult, supporting the normal GSH, ALP, and ACP profiles previously recorded in this group [4,6]. Contrastingly, exposure to individual pesticides—dichlorvos (Group B, Plate 2), dimethoate (Group C, Plate 3), and cypermethrin (Group D, Plate 4)—each elicited specific histopathological changes consistent with oxidative and inflammatory neuropathology. Dichlorvos induced multiform nuclear pyknosis (NP) and granular cell clumping (GC), indicative of apoptotic activity and disrupted neuroarchitecture [3,1]. Dimethoate exposure resulted in oedematous granular layers (OG) and nuclear pyknosis, hallmarks of cytotoxic edema and neuronal apoptosis, consistent with earlier reports linking dimethoate to blood-brain barrier disruption and inflammatory gliosis [5]. Cypermethrin caused glial cell degeneration (GD) alongside nuclear pyknosis, suggesting impairment not only of neurons but also of neuroprotective glial cells, which play a vital role in redox balance and neurotransmitter regulation [8].

Combined pesticide exposures markedly exacerbated neuropathological damage. The dichlorvos-dimethoate group (Group E, Plate 5) demonstrated granular layer oedema (GO), implying additive effects on vascular permeability and cellular fluid homeostasis. This correlates with significantly reduced GSH and ALP levels and heightened ACP activity in this group, reinforcing a model of compound-induced lysosomal stress and membrane dysfunction [9,19]. Similarly, vascular congestion (VC) observed in the dichlorvos-cypermethrin group (Group F, Plate 6) reflects compromised microvascular integrity, possibly due to endothelial oxidative injury and neuroinflammation, as documented in mixed pesticide exposure models [17,2]. Interestingly, Group G (dimethoate + cypermethrin, Plate 7) maintained relatively preserved brain architecture, with intact polymorphic, pyramidal, and granular layers. While biochemical markers indicated stress in this group, the absence of pronounced histopathological damage suggests a comparatively lower interactive toxicity. This may reflect antagonistic interactions, metabolic detoxification capacity, or differences in the blood-brain barrier permeability of these agents [12].

The most severe alterations were observed in Group H (triple pesticide mixture, Plate 8), where pyramidal cell degeneration (PD) and multiform cell degeneration (MD) were extensive. These changes signal widespread

neuronal necrosis, structural collapse, and likely functional deficits in cortical and subcortical processing regions. Such degeneration mirrors the dramatic reductions in GSH and ALP activity and heightened ACP levels observed biochemically, confirming a cumulative or synergistic toxic interaction [18,10]. Collectively, the histopathological data provide morphological validation for the biochemical, enzymatic, and oxidative disturbances induced by pesticide exposure. They highlight the tissue-level consequences of both single and combined pesticide treatments and underscore the neurotoxic potential of pesticide mixtures, particularly in physiologically sensitive states such as the postpartum period, where neuroendocrine vulnerability is heightened [21].

Furthermore, the histopathological findings from Plates 9 to 16 offer microscopic validation of the pulmonary toxicity induced by dichlorvos, dimethoate, cypermethrin, and their combinations in postpartum female Wistar albino rats. These observations mirror the biochemical impairments discussed earlier—particularly in alkaline and acid phosphatase activity—and reveal distinct pathological hallmarks, ranging from vascular and epithelial alterations to inflammatory infiltration and parenchymal destruction. The results also underscore the tissue-specific responses of the lungs to pesticide exposure and the exacerbated toxicity of combined treatments.

In the control group (Group A, Plate 9), the lung tissue displays well-preserved alveolar spaces (AS), intact terminal bronchioles (TE), and minimal perivascular inflammatory infiltration (PI), serving as a baseline of pulmonary integrity. These features suggest optimal lung function and unperturbed histoarchitecture, aligning with enzymatic homeostasis seen in the ALP and ACP profiles of this group [13,6]. Conversely, dichlorvos exposure (Group B, Plate 10) resulted in vascular hypertrophy (VH) and bronchiolar necrosis (BN), indicating compromised vascular tone and epithelial viability. Vascular hypertrophy is often associated with toxicant-induced endothelial activation and remodeling, which can impede perfusion and lead to hypoxic damage [5,19]. The bronchiolar necrosis reflects direct cytotoxic effects on epithelial cells, consistent with oxidative and inflammatory stress pathways triggered by organophosphate compounds [1].

Group C (dimethoate, Plate 11) showed severe pathological lesions, including atelectasis (AT) and interstitial inflammation (EI), both indicative of impaired alveolar ventilation and immune activation. Atelectasis compromises gas exchange and is often linked to surfactant dysfunction and epithelial collapse, which are known effects of dimethoate-mediated oxidative stress [3,9]. The presence of exudates further signals active inflammation and disruption of alveolar-capillary barriers.

Cypermethrin exposure (Group D, Plate 12) also led to notable injury, evidenced by inflammatory exudates (IE), thick bronchiolar secretions (BS), and bronchiolar ulceration (BU). These changes suggest significant irritation of the respiratory epithelium and mucus-producing cells, impairing mucociliary clearance and increasing susceptibility to further damage [15,8]. The ulceration observed reflects loss of epithelial continuity, typically arising from prolonged toxicant exposure.

Among combination treatments, Group E (dichlorvos + dimethoate, Plate 13) displayed regions of apparently normal alveoli juxtaposed with inflammatory exudates and ulceration, revealing localized pulmonary resilience alongside focal damage. The presence of both intact and injured regions highlights a spatially heterogeneous response and reinforces the synergistic potential of organophosphate mixtures in enhancing pulmonary toxicity

[17,2]. Group F (dichlorvos + cypermethrin, Plate 14) showed similar injury patterns, including bronchiolar ulceration with relatively preserved alveoli, indicating that vascular and epithelial effects may dominate over alveolar damage in this combination.

Interestingly, the lungs of rats in Group G (dimethoate + cypermethrin, Plate 15) exhibited largely preserved histoarchitecture, including normal alveoli (AL), terminal bronchioles (TB), and bronchial arteries (BA). This finding is congruent with earlier ALP data showing relatively milder inhibition in this group and suggests possible antagonistic interaction or efficient detoxification capacity that mitigates histological disruption [12,4]. However, latent or functional impairments cannot be ruled out, warranting more sensitive assessments.

The most severe histological changes were found in Group H (triple mixture, Plate 16), with extensive interstitial exudates (IE) and bronchiolar necrosis (BN). These alterations reflect acute and diffuse inflammation, parenchymal injury, and epithelial loss—hallmarks of advanced pesticide-induced lung injury. The findings align with biochemical evidence of marked ALP and ACP disturbances and further confirm the cumulative or super-additive toxicity of combined pesticide exposure [18,10].

In summary, the histopathological evidence reveals that all three pesticides disrupt pulmonary architecture, but mixtures—especially involving dichlorvos and dimethoate—exert synergistic effects that amplify tissue damage, inflammation, and necrosis. These effects are of particular concern in postpartum individuals, where immune modulation and hormonal adjustments may increase tissue sensitivity to xenobiotics [16]. Moreover, the detection of vascular hypertrophy, exudates, and necrosis points to functional impairments beyond morphology, including altered gas exchange, ventilation, and pulmonary clearance.

6. Conclusion

This study demonstrates that exposure to dichlorvos, dimethoate, cypermethrin, and their combinations induce significant oxidative stress, enzymatic disruption, and histopathological damage in the brain and lungs of postpartum female Wistar albino rats. Individually, each pesticide impaired antioxidant defences, disrupted membrane integrity, and triggered cellular injury. However, the toxic effects were markedly exacerbated under combined exposures—particularly in the triple pesticide group—evidenced by profound reductions in glutathione levels, altered phosphatase activities, and severe histological lesions such as neuronal degeneration, vascular hypertrophy, bronchiolar ulceration, and necrosis. These findings underscore the heightened health risks posed by co-exposure to multiple pesticides and highlight the need for stricter regulatory oversight, especially in vulnerable populations such as postpartum individuals. The study reinforces the importance of evaluating the cumulative toxicological burden of pesticide mixtures in real-world scenarios.

7. Future Suggestions

1. Extend the study to include additional biochemical markers such as lipid peroxidation (MDA) and superoxide dismutase (SOD) to provide broader insight into oxidative imbalance.
2. Conduct long-term exposure studies to determine whether the observed neuro-pulmonotoxic effects persist or worsen over time.

3. Investigate possible protective interventions, including antioxidant supplementation or dietary modulation, to mitigate pesticide-induced oxidative stress.
4. Explore molecular pathways, such as mitochondrial dysfunction and apoptotic signalling, to clarify mechanistic bases of pesticide mixture toxicity.
5. Compare the susceptibility of different physiological states (pregnant, lactating, aged, or juvenile rats) to pesticide mixtures for broader toxicological relevance.
6. Translate findings to field-based studies in agricultural communities to validate laboratory results under real-world exposure scenarios.

Declarations

Source of Funding

This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing Interests Statement

The authors declare that they have no competing interests related to this work.

Consent for publication

The authors declare that they consented to the publication of this study.

Authors' contributions

Both the authors took part in literature review, analysis, and manuscript writing equally.

Availability of data and materials

Supplementary information is available from the authors upon reasonable request.

Ethical Approval

All procedures were conducted in accordance with institutional ethical guidelines for animal experimentation and were approved by the University Ethical Review Committee (Approval No.: EMT/2025/012).

Institutional Review Board Statement

Not applicable for this study.

Informed Consent

Not applicable for this study.

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